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# BIOCHEMISTRY

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STANFORD UNIVERSITY



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## IMMUNOGLOBULINS

By the turn of the century, many important features of the immune response had been discovered. An awareness of the protection conferred was fully appreciated much earlier, as evidenced by Thucydides' account of the plague that struck Athens in 430 B.C.:

All speculation as to its origins and its causes, if causes can be found adequate to produce so great a disturbance, I leave to other writers . . . for myself, I shall simply set down its nature, and explain the symptoms by which it may be recognized by the student, if it should ever break out again. This I can the better do, as I had the disease myself, and watched its operation in the case of others. . . .

Yet it was with those who had recovered from the disease that the sick and the dying found most compassion. These knew what it was from experience, and had now no fear for themselves; *for the same man was never attacked twice*—never at least fatally. And such persons not only received the congratulations of others, but themselves also, in the elation of the moment, *half entertained the vain hope that they were for the future safe from any disease whatsoever*. . . .

In characterizing the hope of protection against all disease a vain one, Thucydides clearly appreciated the *specificity* of the protection afforded by the immune system.

## BASIC DEFINITIONS

An *antibody* (or *immunoglobulin*) is a protein synthesized by an animal in response to the presence of a foreign substance. The antibody has specific affinity for the foreign material that elicited its synthesis.

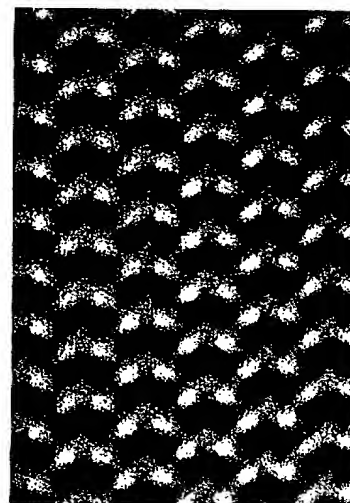


Figure 33-1  
Electron micrograph of a crystal of antibody molecules. [From L. W. Labaw and D. R. Davies. *J. Ultrastruct. Res.* 40(1972):349.]

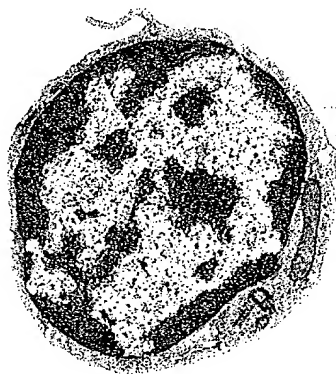


Figure 33-2  
Electron micrograph of a B-lymphocyte.  
[Courtesy of Lynne Mercer.]

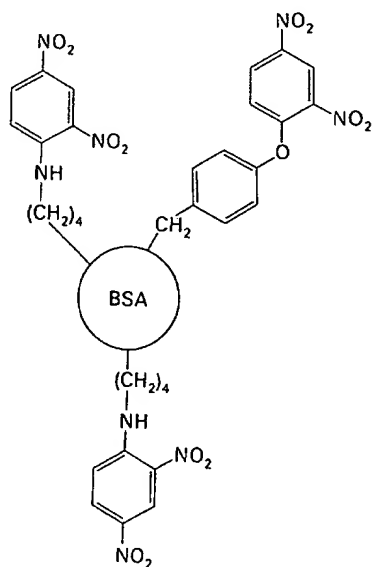
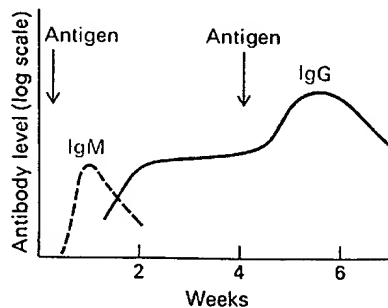


Figure 33-3  
Dinitrophenylated bovine serum albumin (DNP-BSA) is an effective immunogen.

Figure 33-4  
Kinetics of the appearance of immunoglobulins M and G (IgM and IgG) in the serum following immunization.



An *antigen* (or *immunogen*) is a foreign macromolecule capable of eliciting antibody formation. Proteins, polysaccharides, and nucleic acids are usually effective antigens. The specificity of an antibody is directed against a particular site on an antigen called the *antigenic determinant*.

Small foreign molecules do not stimulate antibody formation. However, they can elicit the formation of specific antibody if they are attached to macromolecules. The macromolecule is then the *carrier* of the attached chemical group, which is called a *haptenic determinant*. The small foreign molecule by itself is called a *hapten*.

Antibody molecules are secreted by *plasma cells*, which are derived from *B-lymphocytes*. The other class of lymphocytes, *T-lymphocytes*, mediates the cellular immune response, which complements the humoral immune response carried out by soluble antibody molecules. This chapter will deal with the humoral immune response because much more is known about its molecular basis. However, it is important to note that antibody molecules are just one component of a vast integrated network of molecules and cells that recognizes foreign substances and eliminates them.

#### SYNTHESIS OF A SPECIFIC ANTIBODY FOLLOWING EXPOSURE TO AN ANTIGEN

Animals can make specific antibodies against virtually any foreign chemical group. The dinitrophenyl group (DNP) is particularly effective in eliciting antibody formation and therefore has been used extensively as a haptenic determinant. Anti-DNP antibody can be obtained in the following way:

1. DNP groups are covalently attached to a carrier protein such as bovine serum albumin (BSA) by reaction of fluorodinitrobenzene with lysine side chains and other nucleophiles of this protein (Figure 33-3).

2. DNP-BSA, the immunogen (antigen), is injected into a rabbit. The level of anti-DNP antibody in the serum of the rabbit starts to rise a few days later (Figure 33-4). These *early antibody molecules*

belong to the *immunoglobulin M* (IgM) class and have a mass of nearly 1000 kdal.

3. Approximately ten days after the injection of immunogen, the amount of immunoglobulin M decreases and there is a concurrent increase in the amount of anti-DNP antibody of a different class, called *immunoglobulin G* (IgG), which has a mass of 150 kdal.

4. The level of anti-DNP antibody of the immunoglobulin G class reaches a plateau approximately three weeks after the injection of immunogen. A booster dose of DNP-BSA given at that time produces a further increase in the level of anti-DNP antibody in the rabbit's serum.

5. Blood is drawn from the immunized rabbit. The resulting serum (called an *antiserum* because it is obtained after immunization) may contain as much as 1 mg of anti-DNP antibody per milliliter. The DNP group is particularly effective in eliciting the formation of large amounts of specific antibody. Nearly all of this antibody is of the immunoglobulin G class, the principal one in serum.

6. The next step is to separate anti-DNP antibody from antibodies of other specificities and from other serum proteins. The anti-DNP antibody differs from other proteins in the antiserum in its very high affinity for the DNP group. Consequently, it can be separated by *affinity chromatography*. A column consisting of dinitrophenyl groups covalently attached to an insoluble carbohydrate matrix is formed. The antiserum is applied to this DNP column, which is then washed with buffer. Most proteins in the antiserum flow through because they have little or no affinity for the DNP group or for the carbohydrate support. In contrast, anti-DNP antibody binds tightly to the column. It is released by adding a high concentration of dinitrophenol, which binds to the antibody and thereby displaces it from the DNP groups of the insoluble carbohydrate matrix. A soluble complex of dinitrophenol and anti-DNP antibody emerges from the column. The dinitrophenol is removed by dialysis or by ion-exchange chromatography, which yields a preparation of purified antibody.

#### THE COMBINING SITES OF ANTIBODIES ARE LIKE THE ACTIVE SITES OF ENZYMES

The *combining sites* of antibodies (i.e., their antigen-binding sites) resemble the active sites of enzymes in several ways:

1. The binding constants for haptens have been determined by equilibrium dialysis and spectroscopic techniques. For example, the binding of a colored hapten such as a DNP derivative quenches the fluorescence of the tryptophan residues of the antibody. The